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PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

לשימוש הלשכה
For Office Use

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אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
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נס ציונה 70450

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שמה הוא of an invention the title of which is assignment by the inventors
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(בעברית) (Hebrew)
תולדות פירידין ותכשירי רוקחות המכילים אותם

(באנגלית) (English)
PRIDINIUM DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

מבקש בזאת כי ינתן לי עליה פטנט
hereby apply for a patent to be granted to me in respect thereof.

• בקשת חלוקה — Application of Division	• בקשת פטנט מוסף — Application for Patent Addition	• דרישה רין קדימה Priority Claim		
מבקשת פטנט from Application	• לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
No. מס'	No. מס'			
dated מיום	dated מיום			

• יפוי כח: כללי / מיוחד — רצוף בזה / עוד יוגש
P.O.A.: general / individual-attached / to be filed later-

הוגש בענין filed in case

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היום This 29th בחודש of August שנת 1995 of the year

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טופס זה, כשהוא מוטבע בחותם לשכת הפטנטים, ישמש הוכחה

PRIDIMIUM DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

תולדות פיריידין ותכשירי רוקחות המכילים אותם.

Abstract

A series of carbamates based on the structure of pyridostigmine (PYR) were synthesized and evaluated as potential analgesics and for their ability to treat cognitive impairments associated with cholinergic perturbance such as in Alzheimer's disease (AD). These compounds were examined for their cholinesterase (ChE) inhibition, pharmacokinetics, acute toxicity, lipophilicity and pharmacological effects on the central nervous system (CNS) (i.e. analgesia in mice and reversal of scopolamine induced memory impairment in rats). These compounds are divided into two main groups; Group A: compounds which are based on N-carbohydryl substituted -PYR and Group B which are based on conjugates between compounds of Group A and various sugar residues mediated by a carbohydryl chain. The incorporation of a long enough alkyl chain renders these compounds lipophilic although they contain a quaternary nitrogen in their basic structure. Due to this unique property these PYR-derivatives become permeable through the blood brain barrier. Furthermore, some of the new compounds are 16-18 fold less toxic than their parent compound PYR. Despite their relative low toxicity, these compounds maintain their intrinsic activity as ChE inhibitors and therefore they may be used as centrally efficacious and safe cholinomimetics. Their central activity was demonstrated by their ability to improve retention latency in the rat at doses of 15-20 mg/kg (subcutaneous, octyl-PYR, vide infra) in the passive avoidance test and in their ability to induce analgesia in mice at a dose of 8 mg/kg using the hot plate, tail clip and tail flick analgesic tests. Some of these PYR derivatives are potential safe drugs also for the treatment of other CNS-related diseases such as stroke and diseases involving cholinergic deficieny in the peripheral nervous system (PNS) such as: mysthenia gravis, glaucoma, neurogenic urinary bladder and as a pretreatment of organophosphorus intoxication.

Background of the Invention.

Cholinergic deficiency in the central nervous system is associated with cognitive impairment (1,2,3). In pathological conditions such as Alzheimer's disease (AD) cholinergic deficiency has been consistently observed in discrete brain regions such as the nucleus basalis of Minert and the cerebral cortex and the hypocampus (4,5). Therefore, a rational approach for the treatment of such cognitive impairments would be to elevate the level of acetylcholine in brain. Cholinesterase (ChE) inhibitors such as physostigmine (PHY) and tacrine (THA) has been clinically examined as potential treatment for AD. PHY displayed fairly consistent mild positive benefits (6). Yet, its short half-life and relatively high acute toxicity limits its clinical use. THA, a long-acting reversible ChE inhibitor, is the only drug approved so far by the FDA for the treatment of AD patients (7). However, its hepatotoxicity and peripheral side effects on the GI system such as nausea and vomiting combined with its moderate efficacy only at high doses constitute its major disadvantages (8). Pyridostigmine (PYR) is a reversible ChE inhibitor which is less toxic than PHY and has a longer duration of action than PHY. PYR serves as an effective drug for the treatment of myasthenia gravis (MG) (9). MG is is an autoimmune disease in which the functional nicotinic cholinergic receptor is diminished and it can be treated by prolonging the presence of acetylcholine in the synapse with AChE inhibitors such as PYR (9). PYR is also used for the pretreatment of humans against poisoning by organophosphorus insecticides and nerve agents (6). If PYR was more permeable through the blood-brain barier (BBB) it could have been used also for the treatment of central cholinergic defficiency. However, its quaternary positively charged pyridinium nitrogen limits its permeability into the CNS and confines its use only as a peripheral cholinomimetic drug (6). Earlier efforts were made to develop tertiary analogues of PYR but they displayed lower efficacy than PYR as AChE

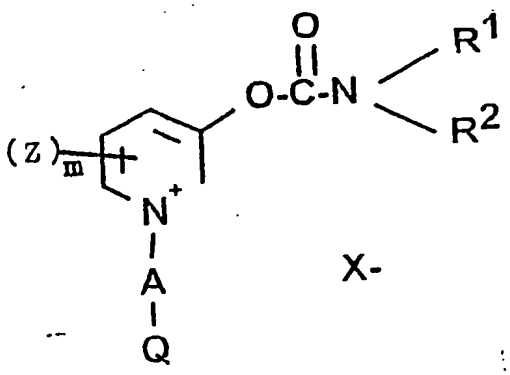
inhibitors (10). The development of PYR derivatives that could cross the BBB, will have longer duration of action and will be less toxic than the existing AChE inhibitors PHY, THA and PYR, will provide a new series of cholinomimetics with improved efficacy and safety.

Summary of the Invention

The molecular design of the new ChE inhibitors which are related to the structure of PYR is based on the attachment of aliphatic chains of various lengths (vide infra) to the quaternary pyridinium nitrogen of PYR. Such carbohydryl chains conjugated to the PYR structure introduce lipophilicity to the resulting new molecule as was shown by the increased distribution coefficient in n-octanol as compared to water (vide infra). According to the three dimensional structure of AChE it was shown that the active site serine residue at position 200 (Torpedo AChE) is located in a 20Å deep narrow gorge lined by many aromatic residues (11). The aromatic residues Tyr337 and Trp84 which reside inside the gorge interact with positively charged quaternary nitrogen of substrates (e.g. acetylcholine) or inhibitors (e.g. edrophonium and PYR) (12). Based on the AChE protein structure and topology, we postulated that a long flexible carbohydryl chain coupled to PYR basic structure will not affect significantly the inhibition potency of the carbamate. On the other hand, due to their increased lipophilicity these compounds could display longer elimination kinetics from blood compared to that obtained for PYR, PHY and other known carbamates (vide infra). Sufficiently long carbohydryl (aliphatic, alicyclic or mixed alipahatic/alicyclic) chains could also serve as spacers or anchors for the attachment of functional groups that may further increase the bioavailability in the CNS and improve the pharmacokinetic profile of the molecule. These functional groups constitute specific carrier recognition factors for various transport mechanisms through biological barriers such as: blood-

brain barrier (BBB), cell membranes and kidney tubuli. As a demonstration for his novel concept we have chosen certain sugar moieties recognized by the glucose transporter. In addition , covalent attachment of lipophilic PYR-derivatives to biodegradable polysacharides via carbohydryl spacers may be used as precursers for sustained release of AChE inhibitors - and thus to further increase their duration of action.

The invention relates to 3-position substituted pyridinium derivatives of the general formula



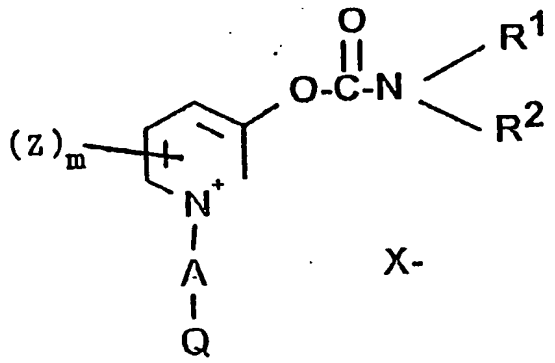
where R^1 is -H, lower alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl,

R^2 is lower alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl,

A is a saturated or unsaturated hydrocarbyl group spacer, and

Z designates dialkylcarbamoyl or lower alkyl, and m is zero or 1.

Q is a transporter recognition moiety adapted to enhance the transport of congeners via biological membranes, which Q entity can optionally be substituted or coupled to a physiologically active acceptable moiety, and where X^- is an anion and to a pharmaceutical composition containing an effective quantity of a compound of the formula:



where R^1 is -H, lower alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl,
R^2 is lower alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl,
A is -H or a saturated or unsaturated hydrocarbyl group, and
Z designates dialkylcarbamoyl or lower alkyl, and m is zero or 1.
Q is -H or a transporter recognition moiety adapted to enhance the transport of congeners via biological membranes, which Q entity can optionally be substituted or coupled to a physiologically active acceptable moiety, and where X^- is an anion.

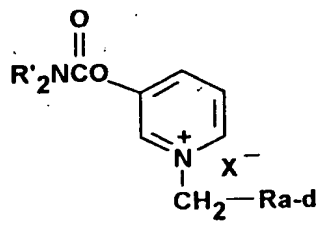
The compounds which are included in this invention are divided into two groups described by the general structural formula in figure 1: compounds of **Group A**, are N-carbohydryl substituted PYR derivatives containing moieties which increase lipophilicity. These moieties include aliphatic chains $(CH_2)_n$ with various lengths of e.g. n= 2- 24 and alicyclic or combined aliphatic and alicyclic hydrocarbon chain. **Group B,** which is described in figure 1, includes compounds which contain PYR as their basic structure and the N-substituted hydrocarbyl chain serves as a spacer arm for the attachment of functional moieties, such as sugar residues, which are recognized by various receptors and membrane transporters.

The PYR-derivatives of this invention can be used as pharmaceutical composition together with known muscarinic and nicotinic agonists, at doses

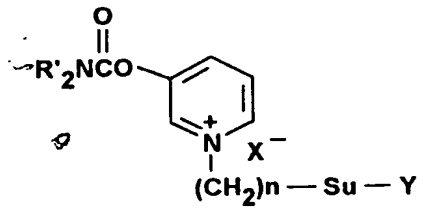
which are lower than those employed for each of the drugs separately. Thus, a synergistic effect is results from the use of such mixtures.

Figure 1.

group A



group B



R' = alkyl or aryl residue

X^- = anionic residue

Su = a sugar residue

Y = phosphoryl or phosphatidyl residue

$R_a = (CH_2)_nCH_3$

$R_b = (CH_2)_nAr$

$R_c = (CH_2)_n$—cyclohexyl

R_d = aryl- alkyl- alicyclic

n = 1 - 23

Alkylations on the 3-carbamoyl pyridine to obtain membrs of group **A** are carried out in similar methods to those described for **2** $_{a-e}$ in the chemical synthesis section (scheme 1). The members of group **B** include also their corresponding precursors which include suitable acetylated or benzylated glycosyl residues as well as inositol derivatives (13). The incorporation of the sugar moiety is achieved, through condensation of the sugar derivative either by its anomeric position as already desribed (see experimental section) or through one of its hydroxyl groups, which is substituted by a suitable leaving group. All the synthetic procedures of the new compounds can be scaled-up using straightforward processes.

The various sugar moieties which could be attached to the molecule via the hydrocarbon chain are:

1. Aldoses which include Aldohexoses: e.g glucose, mannose, galactose, aldopentoses, aldotetroses and glyceroses and their corresponding aldonic and uronic acids.
2. Ketoses which include ketohexoses (e.g. fructose, sorbose), pentoketoses
3. 6-deoxy hexoses e.g. fucose and mannose
4. Alditols which includes manitol and ducitol (C6), ribitol (C5), erythritol (C4), and glycerol (C3)
5. Cyclohexitols (e.g inositol and myoinositol).
6. Ascorbic acid and its derivatives (e.g. dihydro ascorbate)
7. Disacharides (e.g. lactose, maltose and sucrose)
8. Oligesacharides which contain either sialic acid or in the absence of sialic acid.
9. Amino sugars (e.g. glucoseamine, N-acetylglucoseamine)
10. Phosphorylated sugars (e.g. phosphatidylinositol)
11. Polyscharides (e.g. cellulose, amylose) used mainly for the sustained release of the drugs either by covalent coupling or by coating.

Chemical Synthesis

1. General procedure for the preparation of N-Alkyl-3-dimethylcarbamoyl pyridinium bromide (Group A, figure 1).

0.01M of 3-dimethyl carbamoyl pyridine was mixed with 0.015M of the corresponding alkyl bromide in acetonitrile (50cc). Initially an emulsion was obtained particularly in the case of higher alkyl halids. Upon heating the reaction mixture at 80°c. for about 16 hours; the solution gradually became

homogeneous . The work-up included a purification by a silica column chromatography. Elution was carried out with ethylacetate followed by gradient mixtures of chloroform- methanol. All six carbamates of type 2 were obtained as an oily product (see scheme 1).

n.m.r. data of **2a, b, c, d, e:**

2a:

^{1}H-nmr($CDCl_3$): 0.95(t, CH_3); 1.41(m , $C\underline{H}_2CH_3$);
1.99(m,$C\underline{H}_2CH_2N^+$) 3.03, 3.16[2s,$N(CH_3)_2$]; 4.93(t,CH_2N^+);
8.16(m,Hγ); 8.29 (d, Hδ); 9.2 (s, Hα); 9.34(d, Hβ)ppm.

MS (FAB): m/e 223 (M^+).

2b:

^{1}H-nmr($CDCl_3$): 0.83(t, CH_3); 1.30(m, $2CH_2$); 1.32(m, $C\underline{H}_2CH_3$);
2.08(m, $C\underline{H}_2CH_2N^+$); 3.02, 3.15[2s, $N(CH_3)_2$];5.02(t, CH_2N^+);
8.25(m. Hγ);8.38(d,Hδ); 9.4(s, Hα); 9.54(d,Hβ)ppm.

MS (FAB): m/e 251 (M^+).

2c:

^{1}H-nmr ($CDCl_3$): 0.83(t,CH_3); 1.22(m, $4CH_2$); 1.30(m, $C\underline{H}_2CH_3$);
2.03(m,$C\underline{H}_2CH_2N^+$), 3.03, 3.15[2s, $N(CH_3)_2$];5.0(t,CH_2N^+); 8.18(m,Hγ);
8.37(d,Hδ) 9.28(s, Hα); 9.42(d,Hβ)ppm.

MS (FAB): m/e 279 (M^+).

2d:

^{1}H-nmr ($CDCl_3$): 0.85(t, CH_3);1.22[m,$6(CH_2)$];1.32(m, $C\underline{H}_2CH_3$) 2.03(m,
$C\underline{H}_2CH_2N^+$);3.03,3.17[2s, $N(CH_3)_2$];5.0(t,CH_2N^+); 8.15(dd, Hγ),
8.32 (d, Hδ) ; 9.30 (s,Hα);9.46(d,Hβ)ppm.

MS (FAB): m/e 307 (M^+).

2e:

^{1}H-nmr ($CDCl_3$); 0.87(t, CH_3); 1.23(s,8 CH_2); 1.35(m,$C\underline{H}_2CH_3$)
2.02(m, $C\underline{H}_2CH_2N^+$); 3.05, 3.18[2s, $N(CH_3)_2$];5.03(t, CH_2N^+);

8.22(m, Hγ); 8.39(d, Hδ):9.38(s, Hα); 9.51(d, Hβ)ppm.
MS (FAB): m/e 335 (M^+).

2. Preparation of Glycoside-Alkanoyl "Extended Arm" Conjugate (Group B, figure 1).

2.1 Glycosidation: (Compound 5, scheme 2).
A stirred solution of 0.08M 1,8-octanediol in 3:2 (v/v) nitromethane-benzene (90 ml) was boiled until 30ml of the solvent mixture had distilled to ensure complete dehydration and then cooled to room temperature. Mercuric cyanide (0.012M) and 2,3,4,6-tetra-o-acetyl- α-D-glucopyranosyl bromide (0.02M) were added,and the reaction mixture was heated at reflux for 2 hours and afterwards for 72 hours at room tempearture. The reaction mixture was diluted with benzene (30cc), and washed successively with a cold, saturated aqueous solution of sodium hydrogencarbonate and water, then dried with anhydrous sodium sulfate, and finally concentrated in vacuo.

The crude product 5 (scheme 2) was purified on a silica column and eluted with a mixture of dichloromethane-ethylacetate.

^{1}H-n.m.r($CDCl_3$): 1.30 (m,3CH_2);1.57(m, 3CH_2); 2.02 (s,OAc); 2.037 (s,OAc);2.04 (s,OAc);2.08 (s,CH_2OAc);3.48 [m,H_a(C H_2OGlu.)]; 3.63(t, C H_2OH);3.7(m,H-5);3.88 [m,H_b(C H_2OGlu.)]; 4.15(dd, H-6_a); 4.28(dd, H-6_b); 4.48 (d,Hβ);4.99 (dd, H-2); 5.1(t, H-4); 5.21(t, H-3)ppm.

2.2 Triflylation: (Compound 6, scheme 2).
The glycoside 5 (1.5gr) obtained by the procedure described above, was triflylated in chloroform (20ml) by the addition of 2,6-lutidine (1.8cc) and triflic anhydride (1gr). The reaction mixture was stirred at room temperature for 20 hours. Afterwards, the solvent was concentrated in vacuo. The residue was

taken in ether (30cc) and separated from the triflic acid salt. The organic phase was washed with cold water, dried, and concentrated again in vacuo to give a crude product 6 (scheme 2).

2.3 Quaternization:(Compound 7, scheme 2).

A solution of 3-dimethyl carbamoyl pyridine 1 (1.6gr) and cpd. 6 (1.6gr) in acetonitrile (20cc) was stirred at 80°C for 3 hours, and for additional 20 hours at room temperature. The reaction mixture was concentrated in vacuo and purified on a silica column. Elution of the product 7 was carried out with a mixture of cloroform, methanol (4:1).

2.4 Replacement of the anion: (Compound 8a, scheme 2)

Replacement of the triflate anion with Cl^- was achieved by using an anion exchange resin (AG 1-X8, chloride-form) in methanolic solution.

^{1}H-n.m.r. ($CDCl_3$): 1.32(bs, $4CH_2$); 1.48(bs, $C\underline{H}_2CH_20$); 1.99, 2.02, 2.04, 2.08(4s,4-0Ac); 3.05,3.17[2s,$N(CH_3)_2$];3.42, 3.65 and 3.85 (3m, H_a,,H_b,,,CH_2O-glu); 4.03(t,H-5); 4.12(dd, $H6_a$) ;4.25(dd, H-6_b); 4.48(d,Hβ); 4.94(m, H-2&CH_2N^+); 5.07(t,H-4);5.20(t,H-3); 8.16(m, Hγ); 8.33 (d, Hδ); 9.26(s,H-Arα); 9.40(d, H-Arβ)ppm.

MS (FAB) : m/e 625 (M^+).

2.5 Saponification: (Compound 8b, scheme2).

Water (1ml) was added to a solution of 8 (250 mg) in methanol (30cc) and few drops of triethylamine were added to adjust the pH to 11. After 20 hours at room temperature the reaction mixture was neutralized with an acidic cation exchange resin (Dowex 50 H^+).

A crude saponified product 9 was obtained by purification on a small silica column, and elution with methanol. MS (FAB): m/e 458 (M^++1).

3. N-Alkyl- 3-Hydroxy-Pyridinium halides. (scheme 3, 9 a,b,c,d,e,f).

All the 6 members of compound 9 (see scheme 3),were synthesized and characterized in a similar manner to that which was described for 2 a,b,c,d,e derivatives.

9a:

^{1}H-nmr(D_2O): 4.37(s,N^+-CH_3); 7.92(m, H-γ); 7.98(d,H-δ); 8.36(d,H-β); 8.39(s,H-α).

9b:

^{1}H-nmr(D_2O): 0.88(t, CH_3); 1.30(sextet, $C\underline{H_2}CH_3$); 1.93(quintet, $C\underline{H_2}CH_2N^+$); 4.49(t, CH_2-N^+); 7.83(m, H-γ); 8.31(d, H-β); 8.34(s, H-α).

9c:

^{1}H-nmr($CDCl_3$): 0.85(t, CH_3); 1.3(m, $3CH_2$); 2.02(m, $C\underline{H_2}CH_2$-N^+); 4.68(t, CH_2-N^+); 7.86(m,H-γ); 8.55(d, H-β); 8.84(s, H-α).

9d:

^{1}H-nmr($CDCl_3$): 0.84(t, CH_3); 1.24(bs, $4CH_2$); 1.34(bs,$C\underline{H_2}CH_3$); 2.0(m, $C\underline{H_2}CH_2N^+$); 4.65(t, CH_2-N^+); 7.84(m, H-γ); 8.20(d, H-δ); 8.46(d, H-β); 8.92(s, H-α).

9e:

^{1}H-nmr($CDCl_3$): 0.85(t, CH_3); 1.23(bs, $6CH_2$); 1.25(bs, $C\underline{H_2}CH_3$); 2.0(m, $C\underline{H_2}CH_2$-N^+); 4.7(t, CH_2-N^+); 7.86(m, H-γ); 8.18(d,H-δ); 8.47(d, H-β); 8.92(s, H-α).

9f:

^{1}H-nmr($CDCl_3$): 0.86(t, CH_3); 1.22(bs, $8CH_2$); 1.32(m, $C\underline{H_2}CH_3$); 2.0(m,$C\underline{H_2}CH_2$-N^+); 4.64(t, CH_2-N^+); 7.82(m, H-γ); 8.15(d, H-δ); 8.42(d,H-β); 8.86(s, H-α).

4. N-Glucosyloxy Alkyl-3-dimethyl carbamoyl pyridinium (scheme 4, $11a_{1,2};b_{1,2}$).

Bromoalkyl glycosides werre obtained through a glycosidation procedure similar to the one described for 5. Quaternization between compounds $10a_{1,2};b_{1,2}$ with 1 in coventional methods, was carried out and led to the formation of $11a_{1,2};b_{1,2}$ (see scheme 4). These quaternised products were characterised by TLC and NMR.

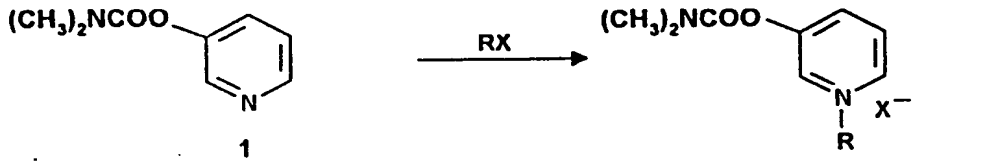
$11a_1$ (decyl):

^{1}H-nmr($CDCl_3$): 1.18(bs,6CH_2); 1.25(m, CH_2CH_2-O); 1.49(m, CH_2CH_2-N^+); 1.93,1.96,1.97,2.01 (4s, 4Ac); 2.98,3.11[2s, N-$(CH_3)_2$]; 2,98, 3.11(2s, N$(CH_3)_2$)3.39,3.64,3.79 (3m, CH_aH_b-0G); 3.95(t,H-5); 4.07(m,H-6_a); 4.2(dd, H-6_b). 4.42(d, H-β); 4.93(m, CH_2N^+,&H-2); 4.99(t, H-4); 5.13(t, H-3); 8.15(m, H-γ); 8.29(d, H-δ); 9.3(s,H-α); 9.44(d, H-β).

$11a_2$ (dodecyl):

^{1}H-nmr($CDCi_3$):.1.24 (bs, 8CH_2); 1.3 (m, 2CH_2); 1.59(m, CH_2); 2.0,2.03,2.05,2.09 (4S, 4Ac); 3.05,3.18 [2s, N-$(CH_3)_2$]; 3.47,3.7,3.87 (3m, CH_aH_b-OG); 4.04(t, H-5); 4.12(dd, H-6_a); 4.27(dd, H-6_b); 4.5(d, H-β); 5.0(m, CH_2-N$^+$,&H-2); 5.08 (t, H-4); 5.21(t,H-3); 8.17(m, H-γ); 8.33(d, H-δ); 9.32(s, H-α); 9.48(d, H-β).

Scheme 1



1

X: Br, I

2 a, b, c, d, e,.

a: R= C_4H_9

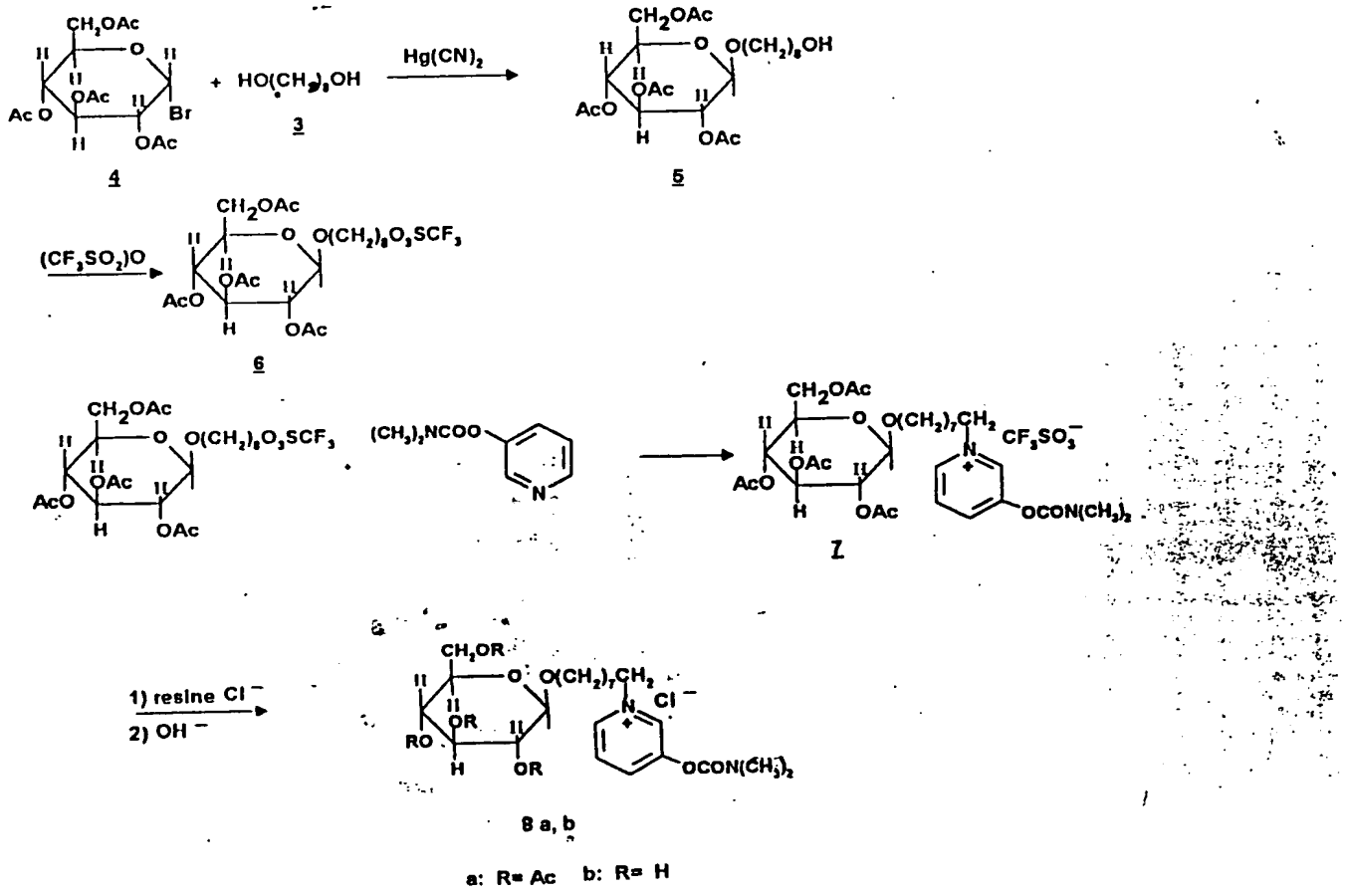
b: R= C_6H_{13}

c: R= C_8H_{17}

d: R= $C_{10}H_{21}$

e: R= $C_{12}H_{26}$

Scheme 2



4

3

5

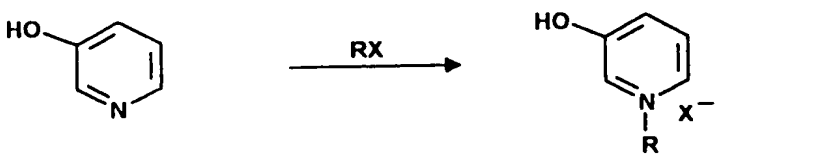
6

7

8 a, b

a: R= Ac b: R= H

Scheme 3



X: Br, I

9 a, b, c, d, e, f.

a: R= CH_3

b: R= C_4H_9

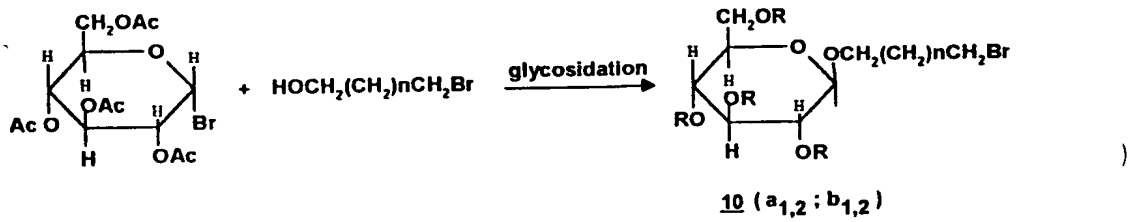
c: R= C_6H_{13}

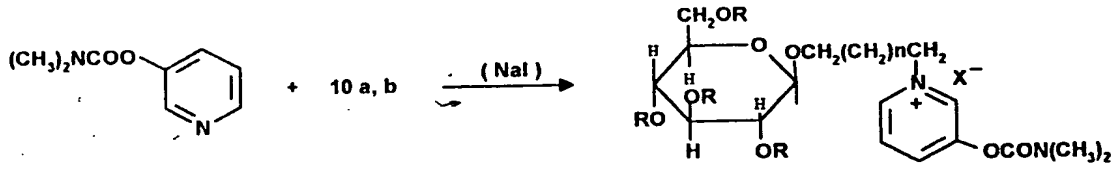
d: R= C_8H_{17}

e: R= $C_{10}H_{21}$

f: R= $C_{12}H_{25}$

Scheme 4





x: Br, I n= 8, 10, etc.

a_1: R=Ac; n= 8
a_2: R=Ac; n= 10
b_1: R=H ; n= 8
b_2: R=H ; n= 10

Kinetics of AChE inhibition and reactivation in vitro.

Carbamates such as pyridostigmine are potent inhibitors of AChE.The mode of AChE inhibition by carbamates is desdribed by the following kinetic scheme:

$$E + I \overset{K_I}{\Leftrightarrow} [E\text{-}I] \overset{k'}{\rightarrow} E_i \overset{k_s}{\rightarrow} E + I'$$

Where E, I, E-I, Ei and I' are the free enzyme, carbamate inhibitor, intermediate reversible complex formed between the enzyme and the carbamate, inhibited enzyme and dimethylcarbamoyl part of the carbamate molecule released spontaneously from the inhibited enzyme, respectively. The inhibition mechanism by carbamates includes the formation of a reversible complex E-I with dissociation constant K_I. The second step is the formation of a covalent conjugate E_i between the dimethylcarbamoyl moiety of the PYR molecule and

AChE, with a first order rate constant k'. Eventually, the inhibited enzyme (Ei) is reactivated spontaneously with a first order rate constant k_s. One can calculate the various kinetic rate constant by following the time-course of AChE inhibition and using the following two equations I and II (14):

I. The approach to steady state:

$$Ln[E_t/E_0 - E_t'/E_0(e/E)_{ss}) = (k'/(1+K_I/I) + k_s)t$$

II. The Steady state equation:

$$(e/E)_{ss} = (k_s/k' + k_sK_I/k') \times 1/I.$$

The bimolecular rate constant of inhibition k_i ($M^{-1}min^{-1}$) is calculated by k'/K_I. The inhibition kinetics was measured with purified fetal calf serum AChE using the Ellman method (21). The various kinetic parameters obtained for AChE inhibition by the various PYR derivatives are summarized in table 1. The values for K_I range between $1.2x10^{-7}$ and $2.3x10^{-5}$M . The spontaneous reactivation rate constant (k_s) obtained for all compounds range between 0.011-0.018 min^{-1}, indicating that the same dimethylcarbomoyl-AChE conjugate was formed upon inhibition by all PYR derivatives. The half-life time values incurred from k_s values are 38-63 minutes as expected from spontaneous reactivation rate of dimethylcarbamoyl-AChE. The overall bimolecular rate constants range between $4.8x10^4$ - $2.9x10^6$ M^{-1} min^{-1}. These results are consistent with our prediction that the addition of hydrocarbyl chain (with or without sugar residue) does not alter the intrinsic activity of the carbamate as an AChE inhibitor.

Table 1: Kinetic parameters of AChE inhibition by PYR derivatives

COMPOUND	K_I M	k' (min^{-1})	$t_{1/2}(k')$ (min)	k_S (min^{-1})	$t_{1/2}(k_S)$ (min)	k_i ($M^{-1}m^{-1}$)
PYRIDO	$5.0x10^{-7}$	0.15	5	0.016	43	$3.0x10^5$
PB	$8.8x10^{-6}$	1.12	0.62	0.012	58	$1.3x10^5$
PH	$2.9x10^{-6}$	0.14	5	0.016	43	$4.8x10^4$
PO	$1.8x10^{-5}$	1.61	0.43	0.014	49	$8.8x10^4$
POGA	$2.3x10^{-5}$	2.11	0.33	0.012	58	$9.2x10^4$
POG	$3.4x10^{-6}$	0.23	3.0	0.012	58	$1.5x10^4$
PD	$3.4x10^{-7}$	0.19	4	0.016	43	$5.6x10^5$
PDGA	$4.0x10^{-7}$	0.19	3.6	0.011	63	$4.7x10^5$
PDG	$8.7x10^{-7}$	0.09	7.7	0.006	110	$1.0x10^5$
PDOD	$2.0x10^{-6}$	0.89	0.78	0.016	43	$4.5x10^5$
PDOGA	$3.6x10^{-7}$	0.69	1	0.018	38	$1.9x10^6$
PDOG	$1.2x10^{-7}$	0.31	2.2	0.059	12	$2.6x10^6$

Acute Toxicity

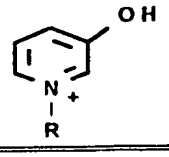
The acute toxicity of the new compounds was determined by i.m. injection in mice and for some of the compounds by s.c. administration in rats. LD_{50} values were calculated according to the Spearman-Kerber method (15). The LD_{50} values obtained in mice for the various PYR-derivatives and their corresponding 3-hydroxy N-alkylpyridinium bromide derivatives are summarized in tables 2 and 3, respectively. Three compounds, PO, PD and POGA display significantly lower toxicity than PYR i.e. 37.6, 36.6, 33.9, respectively, as compared to 2.13 mg/kg (i.m.) obtained for PYR. The LD_{50} values obtained for PO, POGA and PD are 17.6, 16 and 17.2 fold higher than those obtained for PYR, respectively. The subcutaneous LD_{50} obtained for PO in rats (see footnote of table 2) 234.8 mg/kg is 47 fold larger than that for PYR, 5.15 mg/kg. It is pertinent to note that these three compounds are efficaciuos inhibitors of AChE with rate constants which are comparable to those of PYR (table 1). However, their in vivo toxicity is significantly lower than those of all other carbamate derivatives (table 2). The relative low toxicity renders these compounds excellent candidates for further studies as potential drugs for various cholinergic impairment diseases. Following carbamylation of AChE by all PYR derivatives there is a stoichiometric release of the 3-hydroxy N-hydrocarbyl pyridinium moiety. Since these leaving groups are putative metabolites of their parent compounds in vivo we have synthesized these compounds and determined their acute toxicity and inhibitory potency with AChE. The 3-hydroxyalkylpyridinium compounds (the leaving groups) are far less toxic than their parent 3-carbamoyl compounds with LD_{50} values ranging at 600-1000 mg/kg (table 3). The leaving groups could inhibit AChE only at milimolar levels (not shown). The compounds PO, POGA and PD were chosen for further pharmacological studies due to their relative low toxicity.

Table 2: Acute toxicity of PYR derivatives

COMPOUND	LD50 (mice i.m.) mg/kg
PYRIDO *	2.13 (1.9-2.3)
PB	1.74 (0.96-3.2)
PH	6.51 (5.8-7.3)
PO **	37.58 (26.6-52.6)
POGA	33.86 (26.5-43.2)
POG	2.50 (1.7-3.7)
PD	36.59 (25.5-52.5)
PDGA	1.63 (0.74-3.56)
PDG	2.14 (1.9-2.4)
PDOD	1.34 (0.89-2.0)
PDOGA	1.19 (0.85-1.67)

* LD_{50} rat s.c. mg/kg 5.15 (4 - 6.6)
** LD_{50} rat s.c. mg/kg 234.8 (139.7 - 394.4)

Table 3: Acute toxicity of 3-hydroxy N-alkylpyridinium bromide compounds in mice

3-hydroxypyridinium derivatives 	LD_{50} (i.m., mg/kg)
R= Methyl	>1000
R= Butyl	>1000
R= Hexyl	507 (326 - 788)
R= Octyl	421 (299 - 591)
R= Decyl	923 (802 - 1063)
R= Dodecyl	>1000

Pharmacokinetics

One of the disadvantages of existing carbamates such as PYR and PHY is their short duration of action. We expect that PYR-derivatives containing either carbohydryl chains or various sugar moieties coupled to PYR via lipophilic carbohydryl chains will display longer duration of action. Indeed, PO and PD injected into rats caused a dose-dependent inhibition of whole blood ChE

activity that was sustained at 17-47% inhibition level even after 24 hours (Table 4). Data from the literature show that the time-course of PYR elimination from blood is significantly shorter with a half-life of 1.2-1.8 hours following i.v. injection (16).

Table 4: Time -course of blood ChE inhibition in rats following s.c. administration of PO and PD.

TIME (hr.)	% AChE Activity					
	PO (mg/kg)			PD (mg/kg)		
	10	20	40	10	20	40
0	100	100	100	100	100	100
0.25	81	51	-	76	75	68
0.5	83	56	63	62	74	64
1	53	67	63	62	65	55
1.5	88	67		100	75	93
2	67	67	63	81	55	82
2.5	74	59	73	-	-	-
3	72	76	58	-	-	-
4	76	58	-	83	72	100
5	-	58	-	-	-	-
6	-	53	-	86	65	82
24	83	67	-	53	70	69

Distribution in n-octanol/water as a test for lipophilicity.

The permeability of small molecules (up to molecular weight of 1000 dalton) through the BBB is well corellated with their lipophilicity (17). As an indication for the lipophilicity of the compounds we have measured the distribution coeffients of some of the proposed PYR-derivatives in n-octanol and aqueous solution. Concentrations of compounds in both phases was determined by the optical density (OD) at 266 - 272 nm. Calibration curve was performed with PYR in phosphate buffer saline (PBS) pH 7.4, at the range of 0125-25mM. 5ml of PYR solution or PYR-derivative solution in PBS were thoroughly mixed with

5ml n-octanol. Separation was observed following 1 minute centrifugation and the aqueous phase was separated from the organic phase. The absorbance spectrum of each phase was scanned at UV between 240-310nm. The peak value for each compound was used for the determination of its concentration according to the calibration curve obtained with PYR. The distribution coeffients are defined as the concentration ratio in n-octanol/PBS. The same distribution coefficients were obtained for at least two concentrations of PYR-derivatives which differed in two order of magnitude.(0.25-25mM). The distribution coefficients (k) of the tested compounds are summarized in table 5.

Table 5: Distribution coefficients (k) of PYR-derivatives

Compound	k (n-octanol/PBS)
PYR	0.009
PB	0.021
PH	0.149
POGA	0.275
PO	1.680
PD	10.816
PDOD	97.250

As can be seen from the k values presented in table 5, PYR is not soluble in n-octanol whereas dodecyl-PYR (PDOD) is virtually soluble only in n-octanol. Progressive elongation of the alkyl chain attached to the quaternary pyridinium nitrogen increases the lipophilicity of the resulting derivative. These results suggest that the derivatives PH, PO, PD and PDOD could be quite permeable through the BBB. The dual solubility of PH, PO and POGA in water and in n-

octanol (table 5) may be beneficial for transport of the drug from the periphery to the CNS on one hand and for the permeability through the BBB on the other hand. Addition of acetylated glucosyl moiety to the PYR-alkyl derivatives (POGA) reduced lipophilicity of PO from 1.680 to 0.275. However, the k value obtained for POGA lies between the k values of PH and PO suggesting higher BBB permeability than PH. These results indicate that compounds which contain an alkyl chain longer or equal to hexyl may serve as good candidates for centrally active drugs. The tendency to increase lipophylicity with elongation of the chain suggests that a PYR derivative in which the sugar is conjugated via decyl or dodecyl groups may permeate the BBB and be more available to the CNS. Compounds that contain functional groups such as glycosides may be bifunctional in terms of their mechanism of permeability into tthe brain, i.e. utilizing their lipophilicity as well as their endogenous membrane transporter to cross membranal barriers.

Analgesia in mice

One indication for BBB permeability could be central activity of the PYR derivatives. It has previously been shown that analgesia may be induced by cholinomimetics, provided that they penetrate through the BBB. PHY, for example, is a potent analgetic (18) but PYR does not induce general analgesia, probably due to its quaternary nature. We found that the PYR-derivatives PO and PD which are soluble in n-octanol induce analgesia in three different tests in mice - hot plate, tail flip and tail clip (18). All three tests were carried out using male albino CHR mice weighing 25 ± 4 grams. For the hot plate test mice were injected with the tested drug (i.m) or with saline as a control and 15-20 min after the injection were placed on a hot plate (59°C) and the time required for the first response (leg lifting) were measured and recorded as response latency. For the tail clip mice were injected with drugs or saline as described

above and 15-20 min later a paper clip was connected to the tail and time for first response (attempt to remove the clip) was measured and recorded as response latency. In the tail flip test, injections were similar to those described above and the mouse was inserted into 50 ml conic centrifuge tube and the tail left out. The tail was inserted into a water bath warmed to 59°C and the time for flipping the tail to avoid the hot water was measured and recorded as response latency. The mean response latencies obtained for PHY, (0.25 mg/kg) PYR (1.5 mg/kg) and two PYR-derivatives: PO and PD (both 8 mg/kg) are given in Table 6. As shown in table 6, PO and PD were active in all three tests indicating their central analgesic effect.

Table 6: Analgesic Effect of Carbamates.

Compound	Mean Response Latency (sec± sem)		
	Hot plate	Tail clip	Tail flick
Control (saline)	6±1	5±2	2.5±1
Physostigmine	22	26±5	15±7
Pyridostigmine	6±3	14±6	4±2
PO	18	20	12
PD	ND*	20	14

* ND = not determined

Reversal of scopolamine-induced cognitive impairment in rats

Pharmacological manipulation of the central cholinergic system can provide significant changes in performance and behavior. Scopolamine, a centrally active antimuscarinic drug induces a profound decrement in learning and

memory (19). Anticholinesterases can reverse this impairment, provided that they are accessible to the CNS (19). We have tested the efficacy of PYR-derivative PO to reverse scopolamine-induced impairment of aquisition in the passive avoidance behavioral task (20). Rats (Whistar male weighing 225-275 g) were injected subcutaneously with PYR-derivative (PO) or saline and 60 min later animals were injected sc with 0.3 mg/kg scopolamine. Fifteen minutes following the last injection animals were placed in the illuminated compartment of a standard shuttle cage. The latency to enter the dark compartment of the shuttle cage was measured following 3 minutes of aclimation period. Once the animal entered the dark compartment an electrical foot shok was delivered through a metal grid floor. The time required for the rats to cross to the dark compartment was recorded as the initial latency. Twenty four hours later, rats were tested again for the latency to enter the dark compartment. A cutoff of 600 seconds was employed. The time required for entering the dark unsafe compartment was recorded as the 24 hours retention latency. Four groups of 10 rats each were employed in this study as follows: 1) Saline-saline (SA/SA); 2) Saline-Scopolamine-(SA/SC); 3) PO-Saline-(PO/SA); 4) PO-Scopolamine-(PO/SC). Parametric data are expressed as means ± SD and the significance of the differences among the groups were analysed using the Mann-Whitney-U-test. Differences between groups were considered singnificant at $p<0.05$. Table 7 summarizes the means of the initial and the retention latencies obtained for these four test groups at three different doses of PO: 15, 20 and 25 mg/kg. The difference between the tested groups was analysed according to the Mann-Whitney-U-test and presented in table 8. These results clearly demonstrate that PO at 15 and 20 mg/kg could reverse the effect of scopolamine in the passive avoidance test (see SA/SC vs. PO/SC, table 7 and 8). In addition, these results indicate that PO penetrate through the BBB as indeed expected from its distribution coefficient in n-octanol/water. PO at the dose of 25 mg also

reversed the decremental effect by scopolamine, but at this dose certain toxic symptoms were observed (see PO-SA versus SA-SA, table 7 and 8).

Table 7: Retention latency of rats in the passive avoidance test - mean time for n=10 per group measured 24 hours post treatment with PO and scopolamine (SC) and initial test.

DOSE mg/kg	Time (sec)	SA,SA	SA,SC	PO,SA	PO,SC
10	MEAN S.D.	570 79	93 83	486 137	249 183
15	MEAN S.D.	570 79	93 83	491 169	344 236
20	MEAN S.D.	570 79	93 83	600 0	253 190
25	MEAN S.D.	570 79	93 83	381 155	102 107

Table 8: Statistical Mann - Whitney - U - test for the retention latency data presented in table 7.

DOSE (mg/kg)	PO,SA/ SA,SA	PO,SA/ SA,SC	PO,SA/ PO,SC	PO,SC/ SA,SC	PO,SC/ SA,SA
10	-	$p<0.002$	$p<0.02$	$p<0.1$	$p<0.002$
15	-	$p<0.02$	-	$p<0.05$	-
20	-	$p<0.05$	$p<0.05$	$p<0.02$	$p<0.02$
25	$p<0.05$	$p<0.002$	$p<0.002$	-	$p<0.002$

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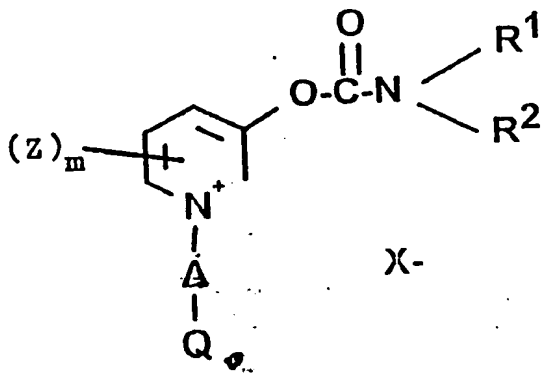
CLAIMS:

1. A 3-position substituted pyridinium derivative of the general formula



where R^1 is -H, lower alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl,

R^2 is lower alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl,

A is a saturated or unsaturated hydrocarbyl group spacer, and

Z designates dialkylcarbamoyl or lower alkly, and m is zero or 1.

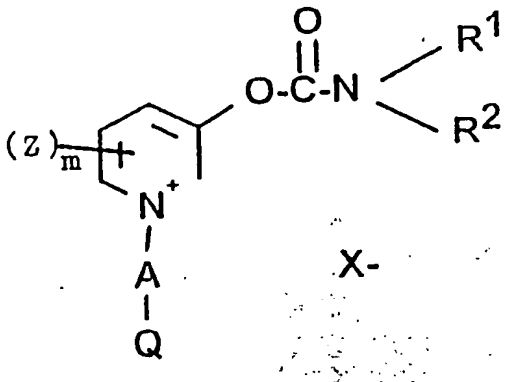
Q is a transporter recognition moiety adapted to enhance the transport of congeners via biological membranes, which Q entity can optionally be substituted or coupled to a physiologically active acceptable moiety, and where X^- is an anion.

2. A compound according to claim 1 where A is $(CH_2)_n$, where n is from 1 to 24
3. A compound according to claim 1, where the Q transporter recognition moiety is selected to enhance the transport of congeners via the blood brain barrier, through cell membranes, through kidney tubuli and through the gastrointestinal wall.
4. A compound according to claim 2, where n is from 4 to 12.
5. A compound according to any of claims 1 to 4, where Q is a sugar moiety.
6. A pyridinium derivative according to any of claims 1 to 5, where the aldose is selected from: glucose, mannose, galactose, aldopentoses,

aldotetroses and glycceroses and their corresponding aldonic and uronic acids.

7. pyridinium derivative according to claim 6, where the ketose is selected from: fructose, sorbose and pentaketoses, where the deoxy hexose is fucose mannitol, or mannose, where the alditol is selected from mannitol and ducitol (C6), rebitol (C5), erythritol (C4), and glycerol (C3), where the cyclohexitol is selected from inositol and myoinositol, where the disacharide is selected from lactose, maltose and sucrose, where the oligosaccharide contains sialic acid, or this is absent, where the amino sugar is selected from glucoseamine and N-acetylglucoseamine, where the phosphorylated sugar is phosphatidylinositol and where the polysaccharide is selected from cellulose and amylose which results in a sustained release drug form. The polysacharides can either be covalently coupled to the PYR-carbohydryl moiety or by physical interaction such as ion-coupling or coating.

8. Pharmaceutical composition containing an effective quantity of a compound of the formula:



where R^1 is -H, lower alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl,
R^2 is lower alkyl, alkenyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl,
A is -H or a saturated or unsaturated hydrocarbyl group, and
Z designates dialkylcarbamoyl or lower alkly, and m is zero or 1.

Q is -H or a transporter recognition moiety adapted to enhance the transport of congeners via biological membranes, which Q entity can optionally be substituted or coupled to a physiologically active acceptable moiety, and where X^- is an anion.

9. A composition according to claim 8 where A is a hydrocarbyl group (CH2)n where n is 1 to 24.
10. A composition according to claim 9 where n is 4 to 12.
11. A pharmaceutical composition of any of claims 8 to 10 for the treatment of, and for the alleviation of symptoms of CNS diseases associated with cholinergic disorders and for the alleviation of side-effects induced by animuscarinic tricyclic antidepressants which comprise an effective quantity of a compound claimed in any of claims 1 to 6 or as defined in claim 8.
12. A composition according to claim 8, for the treatment of Alzheimer disease, tardive diskinesia and effects of stroke.
13. A composition for the treatment of, and alleviation of symptoms of peripheral cholinergic disorders, glaucoma, myasthenia gravis, treatment of urine bladder dome (neurgenic urine bladder) and for the pretreatment of organophosphorus intoxication, comprising an effective quantity of a compound claimed in any of claims 1 to 7 or as defined in claim 8.
14. A pharmaceutical composition of prolonged action, for afflictions in the CNS and periphery, where the pyridynium moiety is coupled to a suitable alkyl chain, a polysaccharide or an oligosaccharide residue.
15. A pharmaceutical composition wherein the pyridinium moiety is coupled to a biodegradable polysaccharide for the slow release of the active component and for use in a biodegradable device for the sustained delivery of carbamates to the peripheral system.

16. 3-positioned substituted pyridinium compounds and compositions containing them, substantially as hereinbefore described and with reference to any of the Examples.
17. Pharmaceutical combinations of the 3-positioned substituted pyridinium compounds and compositions containing them as described in claim 16 together with nicotinic and/or muscarinic agonists which confer higher efficacy in the treatment of cholinergic deficiency diseases.

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